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REVIEW ON ANALYTICAL METHODS DEVELOPMENT AND VALIDATION FOR EZETIMIBE AS AN ANTI-HYPERLIPIDEMIC AGENT

Parth M. Patel*, Smit B. Patel, Tisha S. Patel, Fatema M. Vohra, Priyank G. Patel

India.

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***Corresponding Author**
Parth M. Patel
India.

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ABSTRACT

Ezetimibe is a cholesterol-lowering medication that works by selectively inhibiting the absorption of cholesterol from the small intestine. It is used alone or in combination with other drugs like statins to treat high cholesterol levels and prevent cardiovascular disease. By blocking cholesterol absorption, ezetimibe reduces total, LDL, and non-HDL cholesterol, and can also affect other lipids like triglycerides and HDL-cholesterol.

The present review focuses on the development and validation of analytical methods for the estimation of ezetimibe in bulk drug and pharmaceutical dosage forms. Various analytical techniques reported in the literature, including UV-Visible spectrophotometry, High Performance Liquid Chromatography (HPLC), and High Performance Thin Layer Chromatography (HPTLC), are critically discussed. Emphasis is given to method development strategies, selection of solvents, detection wavelengths, chromatographic conditions, and validation parameters as per International Council for Harmonisation (ICH) guidelines.

Overall, this review confirms that properly developed and validated analytical methods are essential for ensuring the quality, safety, and efficacy of ezetimibe as an antihyperlipidemic agent in pharmaceutical industries.

KEYWORDS: Ezetimibe, Hyperlipidemia, Cholesterol absorption inhibitor, Analytical method development, Method validation, UV–Visible spectrophotometry, HPLC, HPTLC, ICH guidelines.

INTRODUCTION[1-7]

Ezetimibe is a cholesterol absorption inhibitor. Ezetimibe is used alone or combination with diet and other cholesterol-lowering medicines (Eg: fenofibrate, statins) to treat high cholesterol and triglyceride (fats) levels in the blood, in patients with heterozygous familial hypercholesterolemia (hofh). Ezetimibe is an inhibitor of intestinal cholesterol absorption. It is indicated to reduce total cholesterol, low-density lipoprotein (LDL), apolipoprotein B (apo B), and non-high-density lipoprotein (HDL) in patients with primary hyperlipidemia, mixed hyperlipidemia, familial hypercholesterolemia (HoFH), and homozygous sitosterolemia (phytosterolemia). Ezetimibe is a prescription medicine that helps lower the amount of low-density lipoprotein or LDL ("bad" cholesterol) in your blood.

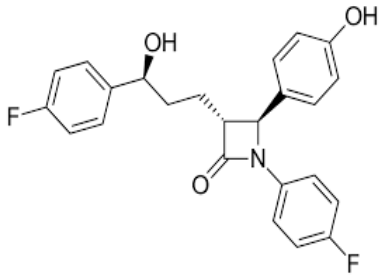
Ezetimibe works by stopping the absorption of cholesterol from the small intestine. Because of this action, it helps lower total cholesterol, LDL (bad cholesterol), and non-HDL cholesterol. To ensure that ezetimibe medicines are safe and effective, proper testing is very important. Analytical methods help to check the quality, purity, and strength of the drug in bulk form and in pharmaceutical dosage forms. Different analytical techniques are used to estimate ezetimibe, such as UV-Visible spectrophotometry, High Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC). These methods are developed and validated according to ICH guidelines to make sure they are accurate, precise, and reliable. In conclusion, well-developed and validated analytical methods are essential for quality control of ezetimibe in the pharmaceutical industry and help ensure patient safety. It was FDA approved in 2002.

DRUG PROFILE[8-13]

History of Ezetimibe

Ezetimibe was discovered in the 1990s and later approved for medical use in the early 2000s. It became popular because it blocks cholesterol absorption in the small intestine, which helps lower LDL (bad cholesterol) levels. Doctors found that ezetimibe works well alone and gives better results when combined with statins.

Structure of ezetimibe



Mechanism of Action

Ezetimibe lowers blood cholesterol by stopping the absorption of cholesterol and plant sterols from the small intestine. It does not affect the absorption of fat-soluble vitamins or other nutrients. Ezetimibe works by blocking a protein called NPC1L1, which helps cholesterol enter the intestinal cells. Normally, this protein carries cholesterol from the intestine into the body. Ezetimibe blocks this process at the intestinal brush border, especially in the jejunum. By stopping cholesterol absorption, less cholesterol reaches the liver. This reduces cholesterol storage in the liver and helps remove more cholesterol from the blood, leading to lower blood cholesterol levels.

Pharmacokinetics

- **Absorption**

Ezetimibe is absorbed after oral intake and changes into an active form. It reaches maximum level in blood in 4–12 hours. It can be taken with or without food.

- **Distribution**

More than 90% of ezetimibe and its active form bind to blood proteins.

- **Metabolism**

Ezetimibe is quickly converted into its active form in the body and does not affect other medicines much.

- **Elimination**

Ezetimibe and its active form stay in the body for about 22 hours. Ezetimibe is mainly excreted in feces, while ezetimibe-glucuronide is removed through urine.

Pharmacodynemic

Ezetimibe helps lower total cholesterol, LDL (bad cholesterol), triglycerides, and other harmful lipids, while increasing HDL (good cholesterol) in patients with high cholesterol. It

works better when used together with statins or fenofibrate than when used alone. In patients with inherited cholesterol problems, ezetimibe reduces LDL cholesterol by about 15–20% and increases HDL cholesterol by 2.5–5%.Ezetimibe should not be used in patients with serious liver problems. In rare cases, muscle pain or muscle damage may occur, especially when ezetimibe is taken with statins.

REVIEW OF LITERATURE

Official method for assessment of Ezetimibe

Sr. No.	Official in	Method	Description	Ref. No.
1.	Indian pharmacopeia (2022) (Volume11)	Liquid chromatography	Stationary phase: A stainless-steel Column (250×4.6 mm. 5 µm), packed with octadecylsilane bonded to porous silica Mobile phase: Ammonium acetate buffer: Water (pH:4.5) (50:50%v/v) Flow rate: 1 mL/min. Wavelength: 210 nm Injection Volume: 20 µ	14

Reported method for assessment of Ezetimibe

Sr. no.	Tittle\method	Description	Ref. no.
UV VISIBLE SPECTROPHOTOMETRY			
2.	Development of UV **spectrophotometric method** for the estimation of ezetimibe from tablet formulation.	**Solvent:** Acetate buffer (pH:4.5) **Wavelength:** 232 nm **Linearity:** 5-30 µg/mL	15
3.	UV and three derivatives **spectrophotometer method** for determination of ezetimibe in tablet formulation.	**Method A:** Quantitative determination of the drug **Solvent:** Methanol **Wavelength:** 233 nm **Linearity:** 6-16 µg/Ml2 **Method B:** First, second and third derivative spectrophotometric method **Wavelength:** 259.5 nm, 269nm and 248nm **Linearity:** 4-14 µg/mL	16
4.	Spectrophotometric Method for the Determination of Ezetimibe in Pharmaceutical Formulations	**Solvent:** Ethanol **Wavelength:** 234 nm **Linearity:** 5-20 µg/mL	17
8.	Analytical Method Development and Validation of Ezetimibe by Using UV-Spectrophotometric	**Solvent:** Ethanol **Wavelength:** 234 nm **Linearity:** 2-12 µg/ml.	18

	Method		
9.	Simple novel UV-spectroscopic method for estimation of Ezetimibe in tablet dosage form	**Solvent:** Ethanol: Acetic acid (90: 10%v/v) **Wavelength:** 252 nm **Linearity:** 2-20 µg/mL	19
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY			
11	Validated **RP- HPLC** Method For Estimation of Ezetimibe in Different Tablet Dosage Form	**Stationary phase**: Phenomenex, (250 x 4.6mm) Luna C-18 column **Mobile phase**: Acetonitrile: Methanol (50: 50%v/v) **Flow rate:** 1 mL/min **Wavelength:** 245 nm	20
12	Development and validation of a reversed-phase **HPLC** method for the determination of ezetimibe in pharmaceutical dosage forms	**Stationary phase**: Kromasil 100 C18 column **Mobile phase:** Water (pH 6.8, 0.05%, v/v 1-heptane sulfonic acid) and Acetonitrile (30: 70%v/v) **Flow rate:** 0.5 mL/min **Wavelength:** 232 nm	21
HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY			
15	HPTLC method for determination of ezetimibe in tablets	**Stationary phase:** Silica gel60GF254 **Mobile phase:** Toluene: Acetone (6 :4%v\v) **Wavelength:** 233nm **Linearity:** 300-200ng\spot	22

CONCLUSION

The development and validation of analytical methods for the estimation of Ezetimibe play a crucial role in ensuring the quality, safety, and efficacy of pharmaceutical formulations. A well-optimized and validated method provides reliable, accurate, and reproducible results that comply with ICH and regulatory guidelines. Various analytical techniques such as UV spectrophotometry, HPLC, and LC–MS have been successfully employed for the quantification of these drugs in bulk and dosage forms. Each method offers unique advantages depending on sensitivity, selectivity, and sample matrix. Method validation parameters—such as linearity, precision, accuracy, specificity, LOD, LOQ, and robustness—confirm the method's suitability for routine quality control and stability studies. Overall, systematic method development combined with thorough validation ensures consistent therapeutic performance of Ezetimibe and supports pharmaceutical research, formulation development, and regulatory compliance.

REFERENCE

1. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, Goldberg R, Heidenreich PA, Hlatky MA, Jones DW, Lloyd-Jones D, Lopez-Pajares N, Ndumele CE, Orringer CE, Peralta CA, Saseen JJ, Smith SC, Sperling L, Virani SS, Yeboah J. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol, 2019 Jun 25; 73(24): 3168-3209. [PubMed]
2. Goldenberg MM. Pharmaceutical approval update. P T. 2013 Jul; 38(7): 389-403. [PubMed]
3. Cleveland clinic "Ezetimibe: Uses and Side Effects" Oct 2025 https://my.clevelandclinic.org/health/treatments/ezeimibe-tablets
4. Nutescu EA, Shapiro NL: Ezetimibe: a selective cholesterol absorption inhibitor. Pharmacotherapy, 2003; 23(11): 1463-74.
5. Brar KS. Ezetimibe (Zetia). Med J Armed Forces India. 2004 Oct; 60(4): 388-9.
6. Lucio Mendes Cabral, valeria Pereira de Sousa and Eduardo Costo Pinto, "Ezetimibe: A review of analytical method for the drug substance, pharmaceutical formulation and biological matrices" *Critical review in analytical chemistry*, **2022;** (5): 1078-1093.
7. Dr. S. Ravi Sankar, Textbook of pharmaceutical analysis; 3rd Edn; Rx Publication, Tirunelveli, 2001; 2-13.
8. Phan BA, Dayspring TD and Toth PP, "Ezetimibe therapy: mechanism of action and clinical update" *Vasc Health Risk Manag.* **2012;** 8: 415-27.
9. Ebmconsult "The mechanism action of Ezetimibe on the inhibition of cholesterol absorption", Sep 2025. https://www.ebmconsult.com/articles/ezetimibe-mechanism-action-inhibitcholesterol-absorption-intestine
10. Kosoglou T, Statkevich P and Johnson-Levonas AO: "Ezetimibe: a review of its metabolism, pharmacokinetics and drug interactions". *Clin Pharmacokinet*, **2005**; 44(5): 467-94.
11. Drug Bank "Pharmacodynamic of Ezetimibe" https://go.drugbank.com/drugs/DB00973
12. Lucio Mendes Cabral, valeria Pereira de Sousa and Eduardo Costo Pinto, "Ezetimibe: A review of analytical method for the drug substance, pharmaceutical formulation and biological matrices" *Critical review in analytical chemistry*, **2022;** (5): 1078-1093.

13. Dr. S. Ravi Sankar, Textbook of pharmaceutical analysis; 3rd Edn; Rx Publication, Tirunelveli, 2001; 2-13.
14. The Indian Pharmacopoeia, Government of india. Ministry of health and family welfare, 9th ed. Ghaziabad: The pharmacopoeia commission; 2022; (2): 1304-1305, 2323-2324.
15. Khemchand Gupta., Indrajeet Singhvi, "Development of spectrophotometric method for the estimation of ezetimibe from tablet formulation." *Int. J. Chem. Sci.,* **2015;** 13(2): 1051-1056.
16. Metreyi Sharma et al, "UV and three derivative spectrophotometric methods for determination of ezetimibe in tablet formulation." *Indian J Pharm. Sci.,* **2008;** 70(2): 258-60.
17. Bh.Ch. Narasimharaju et al., "Spectrophotometric method for the determination of ezetimibe in pharmaceutical formulations." *Biomed. Pharmacol. J.,* **2008**; 1(2): 413- 416.
18. Manjesh Gowda U S et al., "Analytical method development and validation of ezetimibe by using UV-Spectrophotometric method." *Int. J. Pharma. Pharma Res*., **2023**; 26(2): 357-364.
19. Satwashila Shahajirao Kadam et al. "Simple novel UV-spectroscopic method for estimation of Ezetimibe in tablet dosage form." *American. J. Pharm. Health Res.,* **2014**; 2(9): 2321-3647.
20. Prabhat K Shrivastava et al.,"Validated RP-HPLC method for estimation of ezetimibe in different tablet dosage form." *Int. J. Pharmt. Sci.,* **2009**; 1(1): 176-181.
21. R Sistla et al., "Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms." *J. Pharm. Biomed. Anal.,* **2005;** 39(3-4): 22-517.
22. S.S. SONAWANE et al.,"HPTLC Method for determination of ezetimibe in tablets."*Asian J. Chem*., **2007**; 19(6): 4925-4927.